Reply to Reviewer 1

Thank you for your time and comments on our manuscript.

Below are the answers to the raised problems in the manuscript. Corrections will be added in the revised manuscript.

The manuscript presents a metabarcoding and geochemical study of the microbial communities associated with mirabilite deposits in the Izvorul Tăușoarelor Cave (Romania). The authors introduce the term “microbiocosm” to describe the interconnected biotic and abiotic components influencing mirabilite formation. The study combines elemental analyses, microbial diversity profiling, and limited ecological interpretation.

While the topic may be of local speleological and microbial ecological interest, I find that the paper, in its current form, does not meet the standards or scientific scope of Biogeosciences. The study is primarily descriptive, lacks robust quantitative data, and provides limited mechanistic insight into biogeochemical processes. Moreover, there are numerous speculative statements throughout the manuscript that are not sufficiently supported by data. For instance, the absence of sulfate-reducing bacteria in mirabilite samples is over-interpreted as mechanistically significant, yet no supporting metabolic or environmental evidence is provided.

In my opinion, the main limitation is the low number of mirabilite replicates, which should be the focal point of the study. Two samples are insufficient to provide a statistically or biologically meaningful description of the microbial communities involved. The authors should substantially increase the number of replicates if they wish to maintain this focus. In addition, too many functional assumptions about bacterial roles are made based solely on 16S rRNA gene data. This approach cannot robustly infer metabolic functions or biogeochemical activity. To strengthen the manuscript, metagenomic sequencing should be performed, at least on the mirabilite samples. I also recommend quantitative PCR analyses targeting both prokaryotic and eukaryotic (fungal) domains, as fungi are likely to play a significant ecological role in cave environments.

There were two samples, each with three replicates. More samples were taken but sequences could not be obtained. This is a general problem with samples from oligotrophic caves.

We only propose here a mechanism and processes that were not described or mentioned in any of the studies concerning mirabilite, especially that no investigation was done on cave mirabilites. This is an extremely rare mineral in caves worldwide. The scope of the study was to find if bacteria are found in mirabilite, since it is so rare and the study was a part of an exhaustive investigation of cave bacteria from different substrate. We changed the Discussion to add underscore the need for more

investigations and that we only propose a mechanism which might be different under a metagenomic study.

We agree on the importance of fungi but was not the scope of the study. We tried to obtain as many results as possible during the project dedicated to investigating bacteria in caves.

There are also important issues regarding the presentation and interpretation of results. The Results section is difficult to follow and should be completely reorganized. Taxonomic nomenclature and database usage need clarification: please specify the reference database and version used for classification. For example, according to the latest taxonomic updates, Firmicutes are now referred to as Bacillota, and Actinobacteriota as Actinomycetota. I strongly recommend restructuring the Results section hierarchically—starting from higher taxonomic levels (phylum, class) and progressing to the dominant genera in each sample. Consequently, Figure 2 should be redesigned to display phylum- or genus-level distributions, which would improve readability. If the authors wish to retain the ASV-based information, this could be shown as a heatmap or supplementary table. The rationale behind the current Figure 3A–C is unclear, the plots are confusing and provide limited information.

We corrected the name of phyla according to the new taxonomy and reorganized the Results as suggested. We also reorganized Figure 2 for an easier reading. We have also revised Fig. 3 for clarity.

The Discussion is very limited. Only a few microbial groups are addressed, and the manuscript does not sufficiently engage with relevant literature on cave microbiology. The authors should consult and integrate more studies on cave microbial ecology and substantially revise the Discussion in light of the additional analyses recommended above.

We agree that there is huge and relevant literature on cave microbiology but this was not scope of the paper, to discuss the microbiology of this cave, but to discuss the new results obtained on mirabilite that was never investigated in caves.

However, we would be grateful if you could point out some of the literature that might be relevant to this topic.

We added a paragraph explaining the limitation of the study.

The ecological analyses (e.g., beta diversity and PCA) are well presented. However, alpha diversity indices are missing and should be included, as they are essential for describing within-sample richness and diversity.

We added the alpha-diversity indices for a better comparison between samples.

Overall, the topic has potential, but the manuscript requires an extensive and fundamental revision before it could be reconsidered for publication.

Thank you, again, for the suggestions and corrections. In the name of all the authors,
Dr. Oana Moldovan

Reply to Reviewer 2, Dr. Urszula Zielenkiewicz,

Thank you for your time and comments on our manuscript.

Below are the answers to the raised problems in the manuscript. Corrections will be added in the revised manuscript.

The manuscript by Moldovan at al. describes mirabilite deposits obseved in the extreme environment of an oligotrophic Carpathian cave. Research in this field is particularly interesting in terms of the contribution of biological life to shaping the environment, the interrelationships between various chemical and physical factors and organisms. For their research, the authors selected mirabilite, an unusual mineral deposits found in the Tausoare Cave, and focused on the possible interdependent biotic and abiotic mechanisms involved in its formation. As a result of their research, they proposed their own original name to describe this unusual ecosystem.

This is a very interesting work in an area that is of interest not only to a narrow circle of geological science specialists, but to a much wider audience.

A comparison of the mirabilite's microbiomes with those isolated from the immediate vicinity environment, together with elemental analysis, allowed the authors to outline a quite plausible scenario for the formation of this mineral.

The study used modern sequencing technology (giving very reliable results) with subsequent statistical analyses and comparisons to published data from similar environments.

The work is written in easy-to-follow language, and the results are generally presented well graphically.

Thank you for the appreciations!

However, the work also has some shortcomings.

It is unclear to me why the statement 'Using metabarcoding, a new approach to studying complex microbial-driven interactions in caves' (the same in Abstract) is proposed, given that many papers presenting such data for different caves have been published over the last decade.

We meant "mirabilite," and we changed the phrase for clarity.

Although the chemical structure of the mineral mirabilite is generally known, it would be very valuable to add a chemical analysis of the tested samples (in addition to the elemental analysis shown), which would clearly confirm the authors' claim of the presence of sodium sulfate, calcium carbonate, and calcium sulfate in the mineral

mirabilite. It would also strongly support the proposed microbiological process for the growth of mirabilite in this cave.

To confirm the mineralogical composition of the samples, we have performed additional analysis: identification of soluble sulphur content by analyzing the sulphate concentration in 1/10 solid to water leachate, identification of carbonates and sulphate groups using the characteristic bands in the FT-IR spectra and phase identification using X-ray diffraction. Consistent changes were made in the manuscript.

2.4 DNA Extraction

DNA from dripping water should be isolated with more specialised kit.

We used the same kit for all our water samples across all the studied caves, as we had reliable published results. Please see Moldovan, O.T., et al. 2025. Microb Ecol 88, 17; Theodorescu, M., et al. (2023). Microb Ecol, 86,2847-2857; Bogdan DF, et al. (2023) Front. Microbiol. 14:962452.

Minor points:

L.115-116. The description of the sediment samples in this paragraph is unclear. The names of the sediment samples should be consistent with those in Table 1.

The sample names were corrected.

Table 1. The column "Air CO2 (ppm)" is superfluous/needless showing the same value for all samples. This information could be placed in the text.

We added a sentence in the cave description about the CO_2 level and deleted the column in the table.

Figure 2. Ensure that individual graphics are consistent with each other, particularly those representing the same sample categories. Additionally, panel (g) lacks a phylogeny legend. In paragraph 3.1, it should be clearer where the discussed data are presented (i.e. Fig. 2 g, h, not Fig. 2, if water samples are discussed).

We made some changes in Fig. 2 and in the text for precision.

Figure 3 provides the most accurate representation of diversity data. However, some graphs are not convincing due to the way they are presented. For example, panels a, b and c show too many items in the legends, meaning those with lower abundances are not visible in the corresponding graphics.

We changed the mentioned figures.

L.228. The word "concentration" is not proper for class abundance.

Concentration was replaced with abundance.

L.48 .....in salt lakes. mirabilite, also known as... The beginning of a sentence requires a capital letter.

Letter was corrected.

Thank you, again, for the detailed corrections. In the name of all the authors,
Dr. Oana Moldovan